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Bovine viral diarrhoea virus (BVDV) subgenotypes in diagnostic laboratory accessions: Distribution of BVDV1a, 1b, and 2a subgenotypes

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Abstract

The prevalence of bovine viral diarrhoea virus (BVDV) biotypes and subgenotypes was determined from 131 BVDV positive samples from a diagnostic laboratory. The majority of the isolates were from Oklahoma; however, other states including Kansas, Texas, and Arkansas were represented. These BVDV samples were from submissions of 76 live animals and 55 necropsy samples. There were 131 BVDV samples represented by 117 noncytopathic (NCP), 11 cytopathic (CP) and 3 cases with mixed NCP and CP biotypes. The NCP isolates were more common (P < 0.05) than the CP and NCP/CP combination. The BVDV samples were segregated into three subgenotypes by differential PCR and sequencing of a viral genomic region, 5′-untranslated region (5′-UTR). There were more BVDV1b subgenotypes 60/131 (45.8%) than BVDV1a, 37/131 (28.2%) or BVDV2a, 34/131 (26.0%) (P < 0.05). The organ system involvement included the major categories such as respiratory, digestive, mixed/multiple organs, abortions, and persistent infections (PI). All three BVDV subgenotypes were found in persistently infected (PI) cattle and respiratory diseases, both major requests for BVDV diagnosis. Only one of the 131 viruses was genetically similar to the strains present in U.S. vaccines.

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1. Introduction

Bovine viral diarrhoea virus (BVDV) causes infections of domestic and wild ruminants worldwide

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(Baker, 1995). The BVDV infections range from clinically inapparent infections to severe disease involving one or more organ systems. Historically BVDV was associated with digestive tract disease including high mortality. Currently, BVDV is associated more frequently with respiratory disease and fetal infections. The fetal infections with BVDV, dependent on age of fetus when exposed, include abortions, stillbirths, congenital malformations and persistently infected (PI) calves (Baker, 1995). The PI calf occurs when a susceptible heifer/cow is exposed to noncytopathic (NCP) BVDV during pregnancy from approximately 42 to 125 days gestation (McClurkin et al., 1984). The PI calf is born alive, is immunotolerant to the initial virus infecting the fetus, and is a lifelong shedder of the virus (Baker, 1995). PI cattle are likely the most important reservoir/ source of virus to susceptible cattle.

The BVDV can be classified into biotypes and genotypes (Baker, 1995). Biotypes are based on the presence or absence of visible CPE in infected cell cultures, cytopathic (CP) or noncytopathic (NCP) (Baker, 1995). Genotype classification is based on divergence in the viral genome sequences revealed by phylogenetic analysis (Baule et al., 1997; Couvreur et al., 2002; Evermann and Ridpath, 2002; Letellier et al., 1999; Nagai et al., 2001; Ridpath and Bolin, 1998; Ridpath et al., 1994, 2000; Pellerin et al., 1994; Sakoda et al., 1999; Vilcek et al., 2001; Wolfmeyer et al., 1997). The genotypic differences are also supported by antigenic differences (Fulton et al., 1997; Ridpath et al., 1994; Vilcek et al., 2001). Until recent years there were two genotypes (BVDV1 and BVDV2) (Carmen et al., 1998; Pellerin et al., 1994; Ridpath et al., 1994). More recently the BVDV1 and BVDV2 genotypes have been further divided into subgenotypes BVDV1a, BVDV1b, BVDV2a, and BVDV2b in North America (Flores et al., 2002; Ridpath et al., 1994, 2000). In addition, another classification describes 11 genetic groups of BVDV (Vilcek et al., 2001).

The prevalence of the various BVDV subgenotypes and biotypes in the cattle populations is difficult to accurately determine without extensive surveys of cattle throughout a region or a national survey. Often there are instances or case reports of individual animals or groups of animals within a herd/outbreak with a particular genotype/biotype. There are multiple reports in the U.S. where diagnostic laboratory BVDV

isolates have been characterized into genotype/biotype (Evermann and Ridpath, 2002; Fulton et al., 2003, 2000; Tajima and Dubovi, 2005).

The purpose of this study was to determine the biotypes and subgenotypes, BVDV1a, BVDV1b, and BVDV2a in BVDV-positive samples submitted to a diagnostic laboratory in the U.S. Also the distribution of these BVDV biotypes and subgenotypes was correlated by the clinical histories at submission or pathology diagnoses.

2. Materials and methods

2.1. Viruses

One hundred thirty one samples from the Oklahoma Animal Disease Diagnostic Laboratory (OADDL) that were positive for BVDV were tested in this study (Fulton et al., 2002, 2000; Saliki et al., 1997). These were different isolates than those utilized in a prior study (Fulton et al., 2000, 2003) These BVDV positive samples were supernatants from the viral isolations and confirmed as BVDV (Fulton et al., 2002, 2000; Saliki et al., 1997). The BVDV isolates were then inoculated onto MDBK monolayer cultures, 25 cm² flasks with a 1 ml inoculum (1:10 dilution of original samples) and the total volume was 5 ml. The MDBK cultures were observed for 6 days with presence or absences of CPE recorded. The cultures were frozen at -70 °C, thawed and supernatants collected after centrifugation with subsequent storage at −70 °C. Uninoculated cultures were included as controls.

2.2. BVDV genotyping

Phylogenetic comparison of sequences of the 5'-untranslated region of the viral genome was performed to segregate the BVDV strains into BVDV1a, BVDV1b, BVDV2a subgenotypes (Flores et al., 2002; Ridpath et al., 1994, 2000; Ridpath and Bolin, 1998). Sequences from the 5'-UTR were amplified using the following primer set; left primer 5' CAT GCC CAT AGT AGG AC 3' and right primer; 5' CCATGT GCC ATG TAC AG 3'. This primer set will amplify all recognized member species of the pestivirus genus. This primer set will amplify all recognized member species including all classical swine fever viruses, Border disease viruses,

BVDV genotypes 1 and 2 and all subgenotypes there of. Amplification and sequencing was performed as described (Ridpath and Bolin, 1998). The amplicon submitted for sequencing represented a region for analysis of 268 nt sequence. Reference BVDV subgenotypes for the testing and analysis included the three above subgenotypes plus BVDV2b. These strains included: BVDV1a NADL; BVDV1a C24V; BVDV1a Singer; BVDV1b NY-1; BVDV2a 890; BVDV2a 296; BVDV2b VS 63.

2.3. Review of case records

The accession records by the submitting veterinarian were reviewed to determine the clinical presentation/diagnosis for the animal at the time samples were collected for antemortem testing. For those samples derived from necropsy cases, the diagnosis based on the submitting veterinarian or the diagnostic laboratory pathologist was recorded. Also recorded were the location of the animal when sampled, the tissue(s) used for the viral isolation, and the vaccination status (if known). In addition the isolation of bacteria and *Myocplasma* spp. was recorded.

2.4. Statistical analysis

Comparisons were made between genotypes (combining CP and NCP for each subgenotype) and between biotypes (combining types 1a, 1b and 2a for each biotype). For each of the presentation categories, chi-square goodness-of-fit tests were performed to test the null hypothesis that the proportion of CP versus NCP from the samples was equal to 0.5 (Agresti, 1990). This test was repeated for pairwise testing the null hypotheses that the proportions of types 1a, 1b, and 2a were equal to 0.5. All analyses were performed with PC SAS Version 8.2.

3. Results

3.1. Biotypes and subgenotypes

There were 131 BVDV positive samples from the OADDL representing samples of various clinical histories, locations and samples from 1996 to 2002. There were 117 (89.3%) NCP strains, 11 (8.4%) CP

strains and 3 (2.3%) cases where both CP and NCP strains were reported. There were more NCP than CP biotypes and NCP plus CP biotypes (P < 0.05) and both NCP and CP alone were more common than mixed NCP and CP (P < 0.05). Based on the 5'-UTR sequencing there were three subgenotypes detected: 37/131 (28.2%) BVDV1a; 60/131 (45.8%) BVDV1b; 34/131 (26.0%) BVDV2a. The BVDV1b subgenotype was more frequently isolated than BVDV1a or BVDV2a (P < 0.05). The percentage of BVDV1a and BVDV2a were not significantly different from the other (P > 0.05).

3.2. Clinical presentation and necropsy histories

These samples submitted were from 76 live animals (14 BVDV1a, 44 BVDV1b, and 18 BVDV2a) and 55 necropsy cases (23 BVDV1a, 16 BVDV1b, and 16 BVDV2a). The majority of cases were from Oklahoma. There were also 11 isolates from Kansas, 5 from Texas, and 3 from Arkansas. A commercial laboratory provided 30 samples; however, information was not always provided as to location of animal, clinical/necropsy history or vaccination status. Most of the samples were from Oklahoma, Kansas, and Arkansas accessions to the diagnostic laboratory. With the movement of cattle to this region for forage grazing (native pasture and wheat) plus feedlots, the exact origin of the cowherd was not identified in all cases.

3.3. Clinical features

The histories for the samples from live animals and the pathologic diagnoses of the necropsy cases were obtained by review of the submitted information and the final diagnostic laboratory reports. There were instances where no history or diagnosis was provided. Numerous submissions were requests for testing to screen the animals, and also, several requests were for screening embryo transfer recipients. Four infected embryo transfer recipients were identified.

BVDV positives from clinical or necropsy cases with respiratory disease (no other reported organ involvement) were the most common, 48/131 (19 BVDV1a, 17 BVDV1b, and 12 BVDV2a). There were no statistical differences (P > 0.05) among the frequency of BVDV1a, BVDV1b, or BVDV2a for the respiratory cases. There were 20 cases involving only

the digestive tract (6 BVDV1a, 10 BVDV1b, and 4 BVDV2a). Those from multiple organ systems (respiratory and digestive) included 1 BVDV1a, 10 BVDV1b, and 2 BVDV2a. BVDV was isolated from 8 cases of abortion (5 BVDV1b and 3 BVDV2a). PI cattle were identified on samples from live animals with reported two positive tests by viral isolation and/or an immunohistochemistry (IHC) positive ear notch, 1 BVDV1a, 10 BVDV1b, and 5 BVDV2a. It is possible and likely that other PI animals were in this group of 131. Only one test was performed in most cases to isolate virus from the sample submitted for the animal.

There were three cases of possible mucosal disease (MD) in that both NCP and CP BVDV1a strains were isolated from a case with diagnostic tract lesions. Interestingly, there was one case where the CP strain shared genetic identity to BVDV1a C24V which was in the MLV vaccine given 1-month prior.

3.4. Sources of samples submitted for viral isolation

Samples from live animals were primarily serum or blood with an anticoagulant (EDTA); however, there were two cases with tracheal wash samples. Both CP and NCP strains were isolated from the EDTA blood samples; however, no CP strains were isolated from serums.

3.5. Bacterial and Mycoplasma spp. isolations in BVDV-positive necropsy cases

There were 32 necropsy cases with BVDV isolates from respiratory lesions including 15 BVDV1a, 7 BVDV1b, and 10 BVDV2a. For several of the respiratory necropsy cases other agents including bacteria and Mycoplasma spp. were isolated. For the 15 BVDV1a respiratory necropsy cases there were 6 with Mannheimia haemolytica, 4 with Pasteurella multocida; 2 with Histophilus somni, 1 with Archanobacterium pyogenes, and 3 Mycoplasma spp. For the BVDV1b respiratory necropsy cases there were 4 with M. haemolytica, and 2 with Mycoplasma spp. For the 10 BVDV2a respiratory necropsy cases 2 had M. haemolytica, 1 had P. multocida, 1 had H. somni, 2 had A. pyogenes, and 1 had Mycoplasma spp. There were 7/ 32 total respiratory necropsy cases infected with two or more of the bacteria and Mycoplasma spp. listed above.

3.6. Vaccination history and relationship of isolates

There were 17 of the 131 cases with a recorded history of vaccination. The requested information ideally would have indicated the type, modified live virus (MLV) or killed viral (KV) on inactivated vaccine, and dates of the vaccination with number of doses. Where possible the name of the vaccine permitted retrieval of the strain in the MLV vaccine. These strains such as NADL, Singer, C24V, and 296 are important as the BVDV field isolates must be compared to the vaccinal strains, especially with MLV administration occurring just before sample collection or death. There were 11 cases that had received an MLV vaccine with an identifiable strain. There was only one case that had a strain genetically identical for the isolate and the vaccine, a possible MD case with both NCP and CP strains.

4. Discussion

This study represents a survey for three BVDV subgenotypes in diagnostic laboratory BVDV positive samples. While BVDV1a, BVDV1b, and BVDV2a positives were found; there were controls for BVDV2b included. A BVDV2b CP strain has been identified from a feedlot pneumonia necropsy case in an unrelated feedlot study (R.W. Fulton, 2003, unpublished data). There were three other U.S. publications/studies reporting distribution of BVDV1 and BVDV2 with further use of subgenotypes (Evermann and Ridpath, 2002; Fulton et al., 2003, 2000; Tajima and Dubovi, 2005). In a prior study of diagnostic laboratory isolates (Fulton et al., 2000) there were 64/105 (61.0%) isolates classified as BVDV1 with 41/105 (39.0%) classified as BVDV2. In a subsequent study using 41 of the 64 BVDV1 positives, there were 13/41 (31.7%) BVDV1a and 28/41 (68.3%) BVDV1b strains (Fulton et al., 2003). A study of BVDV strains in the Northwestern United States indicated all were NCP with 10/54 (18.5%) BVDV1a, 22/54 (40.7%) BVDV1b, and 22/54 (40.7%) BVDV2 (Evermann and Ridpath, 2002). Most recently a study of 53 BVDV positive samples from bulk milk (16 dairy herds) and 37 BVDV infected cattle reported 26/53 BVDV1b (49.1%), 6/53 BVDV1a (11.3%) and 21/53 (39.3%) BVDV2a (Tajima and Dubovi, 2005). This current study supports other studies that the BVDV is a collection of diverse subgenotypes in the U.S. with the three major subgenotypes, BVDV1a, BVDV1b, and BVDV2a in the cattle population served by this diagnostic laboratory. There is need for vigilance to detect emerging subgenotypes such as BVDV2b, which was recently identified.

The clinical syndromes of the live animals and the involved organ systems confirmed by the pathologic diagnoses at necropsy indicate multiple organ tropism for BVDV ranging from respiratory, digestive tract, fetal (abortion), multiple organ (respiratory and digestive), and PI cases. There was a wide distribution of organ system involvement for all three subgenotypes. There did not appear to be any single tropism for each subgenotype. There was limited evidence of the presence of conditions such as dermatitis or hyperkeratosis; but the BVDV was not isolated from lesion materials in these cases.

Isolation procedures used would not differentiate between acute and PI infections. Thus to ascribe a clinical condition in a PI animal is difficult as suggested (Tajima and Dubovi, 2005). It is noteworthy in this study that there were PI cattle detected with each of the three subtypes, BVDV1a, 1b, and 2a. The PI status was confirmed by multiple sequential viral isolations and/or positive immunohistochemistry tests. However, those cattle with only CP strains suggest these were acute infections as CP strains do not cause PI status.

The role of BVDV in respiratory disease appears to be causing primary infection of the respiratory tract and it is interacting with bacterial infections such as *M. haemolytica*, *P. multocida*, *H. somni*, *A. pyogenes* and *Mycoplasma* spp. This current study supports a prior study with the concurrent isolation of these bacteria (Fulton et al., 2000). Respiratory disease in beef cattle from this region from postweaning stockers and/or feedlots is a major disease. BVDV remains a significant etiologic agent in respiratory disease and a major contributor to its economic effects.

This study underscores the point that efficacious vaccines controlling BVDV1b infections are needed. The predominant subgenotypes in U.S. MLV or KV vaccines are BVDV1a strains (Singer, NADL, C 24V) and BVDV2a strains (296 and 5912). The current study did utilize these strains to distinguish the field isolates from the vaccinal strain in those MLV vaccines used in the cases from which BVDV was isolated. It is important to distinguish MLV vaccine

strains from the field isolates, because MLV vaccines can cause transient infections postvaccination. There is only one licensed U.S. vaccine containing the BVDV1b strain, and it is a KV vaccine (Fulton et al., 2003). Thus, the BVDV1b strains in this study reflect field infections rather than those of vaccine origin.

There are multiple genomic assays to segregate the BVDV into subgenotypes. This study utilized the 5'-UTR as others have reported (Evermann and Ridpath, 2002; Flores et al., 2002; Fulton et al., 2002, 2003; Pellerin et al., 1994; Ridpath et al., 1994; Vilcek et al., 2001; Wolfmeyer et al., 1997; Nagai et al., 2004). For others the E2 gene was used (Becher et al., 1999; Tajima et al., 2001; vanRijin et al., 1997; Nagai et al., 2004) while others utilized the NS3 (Nagai et al., 2004) and Npro (Becher et al., 1999; Nagai et al., 2004). The major subgenotypes appear to be identified regardless of the genomic region from which the primers are selected.

The existence of subgenotypes is a reflection of the diversity that occurs among BVDV. This diversity is also expressed antigenically (Fulton et al., 2003, 1997). Diversity may impact control when vaccines contain subgenotypes different from the major subgenotypes circulating as natural infections in the cattle population. This study confirms that BVDV1b is the most common subgenotype in this region based on diagnostic laboratory BVDV positive cases in the U.S. Also PI cattle were confirmed with the BVDV1b as well as other BVDV subgenotypes. Effective BVDV vaccines should be developed and utilized to control this predominant BVDV subgenotype.

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